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TITLE: CHEMOPROPHYLAXIS AND ANTIDOTAL EFFICACY OF ALPHA-KETOGLUTARIC ACID IN HYDROGEN CYANIDE POISONING

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The purpose of this project is primarily to establish a model system for exposing animals (mice and rats) to hydrogen cyanide (HCN). A chamber has been designed, constructed and tested for the nose-only dynamic exposure of rats to HCN. A chamber of two compartments is available for exposure of mice to HCN after allowing the chamber to equilibrate.

Analytical metnods (microdiffusion and gas chromatography) for the determination of cyanide (CN) levels in blood and chamber air are established and confirmed. A method, employing selective ion electrode, is being used to monitor the test solutions of cyanide salts.

Blood levels of cyanide from rats which had been injected with 2 mg/kg NaCN attained a level of .1-.15 mcg/ml. In other experiments, in which the animals were treated with alpha-ketoglutaric acid (AKG), a proposed antidote for cyanide poisoning, the blood levels of CN increased only to 0.2 mcg/ml, but the animals survived to a total dose of 3.5 mg or 3.5 x the lethal dose of sodium cyanide.

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19. Abstract (continued)

Other studies in mice have included the comparison of CN antagonists: AKG, sodium nitrite, cobalt edetate, and hydroxocobalamin (HCB) at equimolar doses. All antidotes reduced LD_{40} (4.5 mg/kg) and LD_{70} (5.5 mg/kg, i.p.) of cyanide to an LD_{50} at doses of the antidotes of 200 mg/kg; AKG and HCB exhibited very similar potencies.

FOREWORD

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Arthur A. Hulse 7/13/89

PI - Signature Date

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I. INTRODUCTION

A. Nature of the Problem

Hydrogen cyanide (HCN) is recognized as a rapidly acting, highly toxic chemical. Unconsciousness, respiratory arrest or death can occur so rapidly that antidotal intervention is difficult. Therefore, a chemoprophylactic agent which could be administered orally would be highly desirable.

Very little research has been done on antidotes using HCN as the experimental form of cyanide. A real need exists to develop a model system in which antidotes could be evaluated for their effectiveness in antagonizing the toxic effect of HCN. The potential antidotal agent should be evaluated for effectiveness when administered before or after exposure of the test animal to HCN.

Models for study of the toxic effects from the inhalation of HCN have been designed and used. The apparatus of Matijak-Schaper and Alarie (1982) included head only (trachea cannulated and non-cannulated) exposure to HCN, carbon monoxide and low oxygen. Respiration functions were monitored with plethysmographs. An LC_{50} of 166 ppm was reported for HCN in mice. However, this system, as reported, would be partially unsuitable for antidotal research since the animals could not all be removed simultaneously from the HCN and the concentrations of HCN were increased over the exposure time. It would be difficult to assess the differences in treated and non-treated animals.

Two excellent references (Levin et al., 1982; Levin et al., 1983) discuss the technical as well as the toxicological aspects of inhalation of HCN and provide standard conditions for procedures of exposure studies.

Although Ballantyne (1984) did not elaborate on the inhalation method used, he did report that the concentration of cyanide in the blood, at death, of rats which were exposed by inhalation was much less, by comparison, than the concentration of cyanide in the blood of animals that were injected intramuscularly, e. g., 170 mcg/100 ml by inhalation and 746 mcg/100 ml by intramuscular injection.

In addition, there is not a satisfactory chemoprophylactic agent presently available for use against cyanide poisoning. The antidotal regimen of a nitrite and sodium thiosulfate has serious limitations: i.e., slow response, decreased oxygen transport due to methemoglobin formation, and lack of suitability of route of administration. Also, thiosulfate is rapidly excreted by the kidneys. The nitrites are given only by inhalation

(amyl nitrite) or intravenous injection (sodium nitrite). Other antidotes have been proposed in Europe. Hydroxocobalamin (Vitamin B_{12a}) (HCB) has been used in France and is offered in the U.S.A. under an Investigational New Drug by the Rocky Mountain Poison Control Center. However, hydroxocobalamin therapy requires tremendously large dosages which result in some toxicity, and at great expense.

Cobalt ethylenediamine tetraacetic acid, cobalt edetate (Kelocyanor), is available in Great Britain. This chemical is effective but exhibits extensive, unpredictable and serious toxicities.

The methemoglobin former, dimethylaminophenol (DMAP) is a rapid and effective antidote for cyanide poisoning; however, it also has serious, toxic side effects.

Thus, the search for a more adaptable, efficacious antidote for cyanide poisoning continues. Our laboratory has done extensive research with alpha-ketoglutaric acid (AKG). This chemical is equally as effective as nitrite/thiosulfate (Moore et al., 1986). In combination with thiosulfate, AKG is three times as effective as nitrite/thiosulfate administered prophylactically.

B. Previous Work by Principal Investigator

1. Chemoprophylaxis of cyanide poisoning by thiol- and sulfur-containing compounds.

Thiol- and sulfur-containing compounds were investigated for their abilities to prophylactically prevent death from CN poisoning. Of the number of compounds evaluated, methionine, cystine, cysteine and N-acetylcysteine were found to be significantly effective as prophylactic agents against the lethality of cyanide in LD_{90} doses. On the other hand, the sulfhydryl-containing compounds 2,3 dimercaptopropanol, disulfiram and methane sulfonic acid were ineffective in chemoprophylaxis of potassium cyanide (KCN) poisoning (Benet et al., 1983; Benet et al., 1984).

2. Chemoprophylaxis of cyanide poisoning by carbonyl compounds.

The carbonyl compounds pyruvic acid, ascorbic acid, dehydroascorbic acid, pyridoxal hydrochloride, AKG and B-ketoglutaric acid (BKG) were evaluated for their abilities to prophylactically prevent death from potassium cyanide. The studies showed that pyruvic acid, dehydroascorbic acid and AKG had significantly effective prophylactic abilities against CN poisoning, as did orally administered ascorbic acid. Pyridoxal hydrochloride and BKG did not protect mice

against lethal doses of KCN. The LD_{50} value of KCN was increased to 35.0 mg/kg when AKG was administered prophylactically at a dose of 2 g/kg; this represents a six-fold increase in KCN dosage which resulted in an LD_{50} (Aldous et al., 1984).

The hypothesis that A-keto acid binds cyanide and inhibits its availability at a cellular level to the extent that lethality is prevented is supported by in vitro studies reported by our laboratory in correlation with in vivo studies (Norris and Hume, 1986).

3. Additional studies on chemoprophylaxis of CN poisoning by AKG.

In studies done by our laboratory, it was shown that while AKG alone in a dose of 2 g/kg, i.p., is as effective as $NaNO_2$ (100 mg/kg) and $Na_2S_2O_3$ (1 g/kg) in protecting mice against the lethality of CN, a combination of $Na_2S_2O_3$ and AKG protected in a measure three times greater than a combination of NO_2 and S_2O_3 (Moore et al., 1985; Moore et al., 1986). In studies done in our laboratory to evaluate the effectiveness of orally administered AKG, it was observed that all doses of AKG were significantly effective in decreasing the toxic symptoms of CN poisoning as well as reducing the lethality from CN (Dulaney et al., 1987).

4. Studies on prevention by AKG of CN induced inhibition of cytochrome oxidase (cytox).

In both in vivo and in vitro studies it was shown that inhibition of brain cytochrome oxidase activity by cyanide can be prevented by AKG (Norris et al., 1986).

C. Previous Work by Other Investigators

To be of value, treatment for cyanide poisoning must occur immediately after intoxication. A lethal dose of approximately 156 mg of cyanide can be bound when 10% of an adult's hemoglobin is in the metheme state (Baumeister et al., 1975). The optimum therapeutic level of methemoglobin is estimated to be 25%; however, if inexorable manifestations of cyanide toxicity persist, levels of 40% can be induced (Peters et al., 1982). Methemoglobin levels of 30% or less seem to produce no overt symptoms, while levels of 60-70% can be considered to be lethal (Bodansky, 1951).

Kiese and Weger (1965) have recommended the use of aminophenols in cyanide intoxication. 4-Dimethylaminophenol (DMAP) has been shown to produce methemoglobin at a much more rapid rate than sodium nitrite (Way et al., 1984). DMAP has also been reported to have a much reduced action upon circulation and respiration as compared to nitrite or

the cobalt compounds (Kiese and Weger, 1965). However, Kruszyna et al. (1982) maintain that cyanide is released more rapidly from cyanomethemoglobin in animals pretreated with DMAP than in animals pretreated with sodium nitrite. Thus, they reported animals surviving the initial cyanide insult only to die 30-60 minutes after cyanide injection.

Cobalt edetate has been suggested as treatment of choice in cyanide poisoning (Lancet, 1977). Cobalt edetate (Kelocyanor) is the preferred cyanide antagonist in Europe (Way et al., 1984). The efficacy of cobalt compounds in the protection against cyanide intoxication has been known for many years and was reported by Way et al. (1984) and by Lang (1895). This agent acts by expeditiously chelating tissue-bound and free plasma cyanide (Peters et al., 1982). Its main advantage as a cyanide antidote is that it does not affect the oxygen-carrying capacity of the blood as do the methemoglobin-forming agents. Sodium thiosulfate has been reported to cause a more-than-additive increase in the antidotal effectiveness of the cobalt chelators (Frankenberg and Sorbo, 1975). The main disadvantage of the cobalt chelators is that they may cause ventricular arrhythmias (Way et al., 1984). Anaphylactoid reactions have also been disclosed in cobalt treatment, such as neurotic edema of the face and neck (McKieran, 1980). Cobalt edetate treatment can cause gastrointestinal hemorrhage, hypotension with transient tachycardia, skin rash, and vomiting (McKieran, 1980).

Hydroxocobalamin has been proposed for use in cyanide poisoning (Mushett et al., 1952; Delga et al., 1961). Its effectiveness appears to be due to its ability to bind cyanide, resulting in the formation of cyanocobalamin (Peters et al., 1982). Ivankovich et al. (1980) report that the dose of vitamin B_{12a} necessary to provide protection against cyanide intoxication in the dog was of sufficient quantity to produce a reddish skin discoloration. They also reported that the animals were not maintained in a viable cardiovascular state by hydroxocobalamin therapy.

Cyanide is a potent nucleophile and may react with carbonyl groups to form cyanohydrins (Morrison and Boyd, 1973). Green and Williamson (1937) ascertained that pyruvic acid, an a-keto acid, reacts with cyanide in vitro to form pyruvic acid cyanohydrin. Cittadini et al. (1972) have shown that sodium pyruvate is effective against cyanide intoxication. Way et al. (1984) maintain that, since this compound is actively transported, it is more likely to be distributed to areas of the body wherein cyanide is localized. However, sodium pyruvate affords only minimal protection against the lethal effects of cyanide, and alone is much less effective than either sodium thiosulfate or sodium nitrite (Schwartz et al., 1979).

K. P. Ivanov of the I. P. Pavlov Institute of Physiology, USSR, in 1959 reported on the effectiveness of elevated oxygen pressure on animals poisoned with potassium cyanide. This work was complemented by studies on the protective effect of oxygen against cyanide intoxication performed by Way et al. (1966) in the United States. Way et al. (1966a) found that oxygen exerted only a minimal, if any, protective effect against cyanide intoxication. However, this group discovered that oxygen prophylactically increased the antidotal efficacy of sodium thiosulfate (Way et al., 1966b) and, more important, therapeutically enhanced the antidotal efficacy of sodium thiosulfate (Sheehy and Way, 1968). Although oxygen enhanced the antidotal efficacy of sodium thiosulfate to a "minor degree," it provided potentiation of the efficacy of the antidotal combination of sodium thiosulfate and sodium nitrite (Way et al., 1984).

II. PURPOSE OF THE PRESENT WORK

The purpose or objective of the present work is primarily to establish a model to be used to evaluate antidotes in animals exposed to gaseous cyanide (HCN). Secondarily, the present work should also result in the establishment of the model for exposure in which methods to monitor heart rate, blood pressure and respiratory rate can be incorporated into this model.

Once the model for exposure of animals is established, potential antidotes can be evaluated. These potential antidotes can be administered orally via stomach tube, intravenously or intraperitoneally in order to evaluate their effectiveness against the toxic effects of gaseous cyanide.

Potential antidotal combinations can also be evaluated against gaseous cyanide.

It is important that a hydrogen cyanide model be developed for this form of cyanide because, although most of the literature that has been published has involved cyanide salts, the potential exists for the use of HCN by a foreign power or by terrorists against the U.S.

III. GENERAL METHODS OF APPROACH

In establishing a model using a gas (HCN) which is highly toxic and rapidly active, the first task was to develop safe working conditions. Thus, we hope to renovate an existing exhaust hood. The renovation of this hood would involve (1) channeling exhaust from this hood directly to the outside, on the roof (this system would be independent of the present exhaust system); and (2) providing that the exhaust from this hood would be scrubbed of cyanide prior to emission to the atmosphere. As of this date, this work is

in progress but has not been completed. This delay has prevented us from developing the model.

Once the model is established, rats can be exposed to measured concentrations of HCN for definite time intervals. This will result in an accurate dosing of the animals and allow good reproduction of experiments. Cannulations of femoral vein and artery will provide access to blood samples and allow monitoring of cardiac and respiratory parameters. Respiratory parameters will be accomplished by plythsmograph or needle electrodes. Blood samples are being analyzed for cyanide content. The ability to monitor heart rate and blood pressure and respiration rate will allow us to monitor the toxic effects of cyanide more closely so that antidotes may be evaluated for their effectiveness in animals which have already been exposed to cyanide.

IV. ESTABLISHMENT OF APPARATUS NECESSARY TO EXPOSE ANIMALS TO HYDROGEN CYANIDE

A. Preliminary Work on Cyanide Exposure Chamber

Experiments in which HCN was produced in a static test chamber gave nonreproducible results. NaCN was allowed to react with an excess of H_2SO_4 in a sealed chamber equipped with a sample port and fan. Varying the amount of NaCN (0.1 g, 0.2 g, 0.45 g, and 0.908 g NaCN) with excess H_2SO_4 produced HCN, but in unpredictable amounts. Relocation of the HCN generator outside the chamber did not give better results.

Other tests using a mixture of 1.02% by volume HCN and helium in the chamber did not give any better results.

Concentrations of HCN in the static chamber were finally determined by use of a flow meter and U-tube in a dry ice-acetone mixture. At a rate of 200 ml/min, .05 g of HCN was collected in the U-tube after 10 minutes. Calculations showed the concentration of the gaseous HCN to be 13,448 ppm. The CN electrode once again failed to indicate the correct value.

Based on the prior experiment, it was calculated that in 20 seconds 1.67×10^{-3} g HCN or 4.94×10^{-4} g/L would be induced in the static chamber, which had a volume of 3.373 liters. The concentration of HCN in the chamber would be 448 ppm.

Run No.	No. Mice	Time in sec. of HCN flow at 200 ml/min	HCN Conc. (ppm)	LD at 5 min
1	5	20	448	0
2	5	25	560	100
3	4	22	493	75
4	5	20	448	0*

* = LD (Lethal Dose) after 10 minutes.

B. Design of the Inhalation Chamber for Exposure of Mice to Hydrogen Cyanide

Figure 1 depicts the dynamic inhalation chamber which was designed to expose mice to hydrogen cyanide. The chamber employed was rectangular in shape, having the dimensions of 16.8 inches x 12 inches x 4.75 inches. The chamber was divided into two compartments which were separated from each other by a sliding door. The outer chamber encompassed 47 square inches of floor space, while the larger inner chamber encompassed 144 square inches of floor space. The outer chamber served as a holding station for the animals immediately prior to their introduction into the inner chamber. Animals were placed into the holding chamber when the inner compartment's atmosphere was determined to contain the desired concentration of each gas. After the inhalation chamber's outer door was closed, the inner sliding door was opened, and the animals were immediately introduced into the inner chamber. The inner chamber was sampled subsequent to the introduction of the animals (and closing of the sliding door) in order to measure any change in gas concentration which might be attributed to the introduction process itself. Intermittent gas sampling was performed throughout the exposure period to assure that the particular gas mixture desired remained unchanged throughout challenge. A fan installed in the rear of the inner chamber aided in the maintenance of a homogeneous gas mixture (with no dead space in the chamber).

C. Design of the Inhalation Chamber for Exposure of Rats to Hydrogen Cyanide

Figure 2 depicts the dynamic inhalation chamber designed for the exposure of a single rat to hydrogen cyanide. The chamber is rectangular in shape, having the dimensions of 25.5 cm x 30.5 cm x 25.5 cm. The chamber is fitted with inlet and outlet ports for the administration of hydrogen cyanide, a sample port, circulating fan, rat immobilizer sleeve, and door.

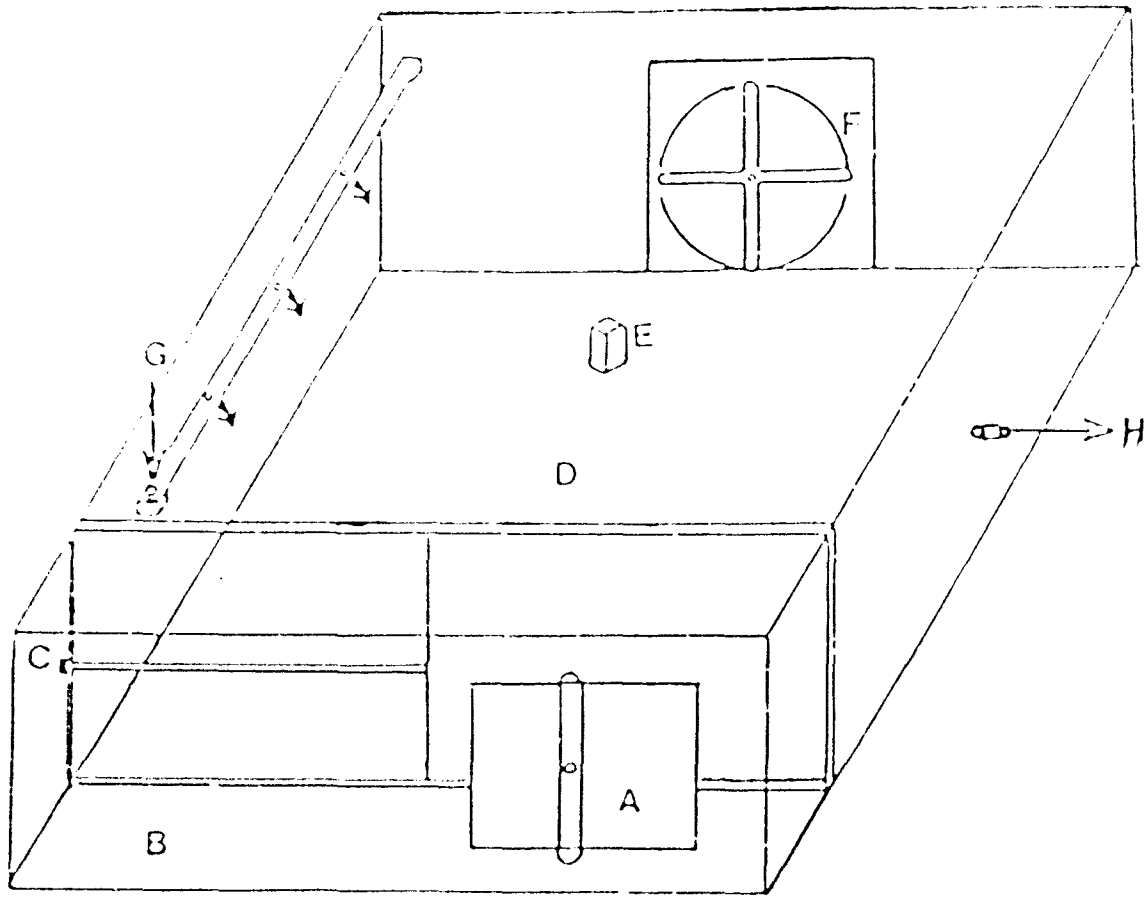


Fig. 1: Design of the inhalation chamber for exposure of mice to hydrogen cyanide.

A. door through which the animals are introduced into the dynamic inhalation chamber; B. outer holding chamber; C. partition between the inner and outer chambers which contains a sliding door; D. the inner chamber; E. gas sampling port; F. fan; G. gas inlet; H. gas outlet

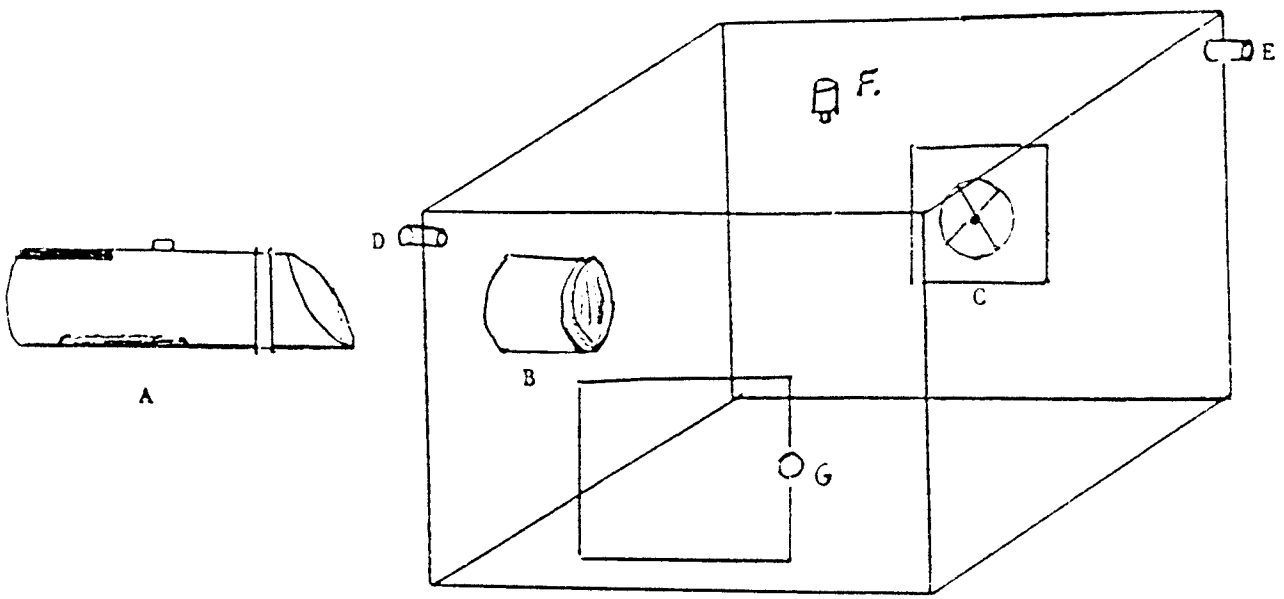


Fig. 2: Design of the Inhalation Chamber for Exposure of Rats to Hydrogen Cyanide. This chamber is designed for exposure to hydrogen cyanide and monitoring cardiopulmonary effects. A. rat immobilizer; B. sleeve for rat immobilizer; C. circulating fan; D. gas inlet; E. gas outlet; F. sample port; G. door

The rat immobilizer is made of a 7.5-cm-in-diameter aluminum tube. It is fitted with dual O-rings which are matched to produce a tight fit into the rat immobilizer sleeve. The immobilizer has grooves at the top and bottom for catheter lines and monitor lines. The immobilizer is constructed so that a circular opening of 2.0 cm in diameter is located 1.0 cm forward of the O-ring. The small opening allows only a portion of the rat's nose to be exposed to the hydrogen cyanide, thereby reducing the area of hydrogen cyanide absorption through the skin.

The hydrogen cyanide is introduced into the chamber through the inlet port. A sample port is provided, allowing the contents of the chamber to be sampled at any time for hydrogen cyanide analysis. When the desired concentration of hydrogen cyanide is reached, the rat, already contained in the immobilizer, is placed into the immobilizer sleeve. Thus, the animal can be exposed to a known concentration of hydrogen cyanide for a determined length of time in order to determine LC values. In addition, when the rats are catheterized, cardiovascular variables can be monitored and blood samples can be collected at any time for analysis of cyanide. All gas exiting the chamber via the exit port is subjected to a sodium hydroxide scrubber before being exhausted through the hood.

V. ANALYSES OF BLOOD FOR CYANIDE CONTENT

A. Analysis of Blood Samples for Cyanide by Microdiffusion

A modification of the method of Feldstein and Klendshoj (1954) has been re-established in our laboratory.

Sodium hydroxide solution is placed in the center well of a Conway diffusion cell. The blood sample is then placed in the outer compartment, acidified with sulfuric acid and the cell sealed for 3-4 hours. A sample is taken from the center compartment, buffered with phosphate and allowed to react with Chloramine T for 2-3 minutes. After the addition of a pyridine-barbituric acid solution, the mixture is allowed to stand for 10 minutes and placed in a visible spectrophotometer, and the absorption at 580 nm is recorded. Calculation is used on a response factor derived from a standard curve.

B. Determination of Whole Blood Cyanide Concentrations by Gas Chromatography

One-tenth of a milliliter of concentrated phosphoric acid was placed into a one milliliter silanized reaction vial. The vial was subsequently capped, and two-tenths of a milliliter of heparinized blood was injected through the

septum. The acid-treated blood samples were vortexed for 30 seconds and then were placed into a 60°C water bath for 60 minutes. Five hundred microliters of vial headspace were injected into a Hewlett-Packard series 5880A gas chromatograph equipped with a nitrogen phosphorous detector.

C. Analysis of Cyanide by Ion-Selective Electrode (The Orion Research, Inc.)

Cyanide Electrode (9406BN) was tested under laboratory conditions to determine whether it would perform satisfactorily in determining sodium cyanide concentrations. After a 1:100 dilution of the sodium cyanide solution, and without a large excess of sodium hydroxide solution to stabilize the mixture, excellent results were obtained. Typical results are shown below.

NaCN Solution g/L	CN^- Conc. 1:100 Dil. g/L	Calculated Meter Reading PPM	Actual Meter Reading PPM	(±)
1.6	8.49×10^{-3}	8.49	9.42	0.93
0.009	4.78×10^{-5}	0.0478	0.0323	0.0155
0.005	2.65×10^{-5}	0.0265	0.00518	0.0208
0.003	1.56×10^{-5}	0.0156	0.00162	0.01398

$$\underset{\text{mw = 49 g}}{\text{NaCN}} \xrightarrow{\text{1.6 g/L}} \underset{\text{mw = 26 g}}{\text{Na}^+ + \text{CN}^-} \quad \text{.0849 g/L}$$

ppm = g/L x 1,000

VI. DETERMINATION OF LD_{50} VALUES FOR POTASSIUM CYANIDE WITH AND WITHOUT ANTIDOTES

Groups of animals (10 per dose) were administered various doses of cyanide in order to generate a lethality curve for each pretreatment protocol. Volumes of the injected solutions were kept small (0.1 ml/10 gram body weight), and the cyanide was always injected antipodal to the peritoneal region into which the cyanide antagonists had been injected, in order to decrease any likelihood of intraperitoneal binding between the cyanide and cyanide antagonists. LD_{50}'s were determined over a period of 12 hours after cyanide challenge.

All LD_{50} values were determined by Statistical Analysis System (SAS) utilizing the methods of Finney (1971) or Litchfield and Wilcoxon (1949). A significance of $p<0.05$ is acceptable.

VII. COMPARISON OF EFFICACY OF ANTIDOTES

The efficacies of several antidotes of cyanide were of particular interest, when compared on a molar basis.

Three different doses of sodium cyanide solution were injected each day of experimentation. The sodium cyanide is calculated on a CN basis for LD_{40} (6.0 mg/kg), LD_{60} (6.3 mg/kg) and LD_{80} (7.1 mg/kg). Injections were made on a 0.1 ml/10 gram weight basis intraperitoneally in the left abdomen.

To evaluate antidotes in their ability to prevent symptoms and lethality of cyanide, injections of the respective antidotes were made at different doses (100, 200 mg/kg) into the right abdomen, contra to the injection of sodium cyanide solution. Animals were observed for onset of symptoms, severity of symptoms and death. Results were calculated according to the statistical methods which can be found in the section on the LD_{50} determination of cyanide.

VIII. PROCEDURE FOR CATHETERIZATION OF RATS

Sprague-Dawley rats, 350-400 grams, (body weight) are anesthetized by the administration of halothane via an inhalation apparatus. Cannulas are placed in the left femoral artery and vein. The cannulas are then run up to the nape of the neck out through an incision which prevented the animal from biting or chewing the cannula. The cannulas are flushed with heparin/saline to maintain potency. Injections of cyanide solutions or antidote can be made through the venous catheter. Also, blood samples can be collected readily for cyanide analysis. The arterial cannula can be connected to the pressure transducer for monitoring heart rate, mean blood pressure and blood pressure.

IX. PROCEDURE FOR MONITORING CARDIOVASCULAR PARAMETERS

Rats, Sprague-Dawley, weight 300-350 grams, are cannulated surgically as described previously. One day after cannulation, the catheters are tested for patency. If patency and animal conditions are acceptable, one animal is placed in the rat immobilizer, which allows access to the catheters. The arterial catheter is connected to the pressure transducer via a polyethylene (PE) tube. The transducer is connected to the model 7 Grass Multichannel polygraph.

The polygraph is calibrated to record mean blood pressure (MBP), blood pressure (BP), and heart rate (HR) simultaneously.

A control non-treated response is recorded. A solution of sodium cyanide is injected (2 mg/kg over 22 seconds) through the venous catheter. MBP, BP and HR are monitored for the toxic effects of cyanide.

The severity and extent of the toxic effects on MBP, BP and HR can be noted. Then the effectiveness of the antidotes in preventing or alleviating these toxic symptoms can be noted and recorded.

This system allows the monitoring of cardiovascular parameters in the conscious animals. It is recognized that cyanide exerts principle toxic effects on the heart which can be monitored. This procedure also allows the evaluation of an antidote administered after the toxic effects of cyanide are present. This is most important if antidotes are to be developed for treating the cyanide intoxicated patient.

X. CONCLUSIONS

Year 1

Alpha-ketoglutaric acid (AKG), hydroxocobalamin (HCB), dicobalt edetate (Kelocyanor) and sodium nitrite were compared when administered to mice 15 minutes prior to i.p. injection of a solution of sodium or postassium cyanide. The results are shown in Table 1 and Figures 3 and 4.

In Table 1, at the LD_{40} and LD_{70} of sodium cyanide and an antidote ratio of 4 or 5 to 1, no deaths were recorded with any of the antidotes used in these studies. AKG was shown to be as effective as sodium nitrite, dicobalt edetate or hydroxocobalamin at these dosages of sodium cyanide.

In Figure 4, AKG is shown to increase the LD_{50} of sodium cyanide from 6.47 to 10.97 at a dose of 200 mg/kg i.p. These results can be compared to HCB at 9.70 mg/kg as approximately the same; also, when sodium thiosulfate is added to AKG and HCB, the LD_{50} is increased to 26.05 and 25.85 mg/kg, respectively. It is apparent from these results that AKG and HCB are quite comparable in efficacy as antidotes for the toxic effects of cyanide.

Blood cyanide levels were obtained after intravenous administration of 0.5, 1.0 and 2.0 mg/kg of sodium cyanide. In Figure 5, the single value for KCN alone represents a blood sample collected at the time of death of the animal.

It can be concluded from the data in figures 6 and 7 that the CN concentration increases in the AKG-treated animals to a level greater than 1.86 mcg/ml at one point, and to a level less than 1.0 mcg/ml for the other samples.

The animals survived until a total of 3.42 mg of sodium cyanide had been administered. In the control group, no animals survived the initial 2 mg/kg dose. It is concluded that AKG does antagonize cyanide effectively when administered after the cyanide has been given.

Cardiovascular monitoring has been initiated in our laboratory. Catheterized rats have been used in these experiments. The arterial catheter is connected via transducer to a Grass model 7 polygraph. A typical control recording is observed in figure 8, in which heart rate, mean blood pressure and blood pressure are monitored. A solution of sodium cyanide at a dose of 2 mg/kg is administered through the venous catheter until the blood pressure decreases and heart rate increases (a typical response to cyanide toxicity). At this point, additional AKG doses (0.5 ml) of AKG can be administered until the animal recovers. This procedure is repeated until the animal deteriorates and is then killed or dies.

In summary, the mechanisms are in place to accomplish much in the area of antidoting cyanide poisoning. Not only can we learn much about the mechanism of cyanide poisoning, i.e., cardiovascular effects, lethal blood cyanide levels, and blood gas effects, but also, antidotes can be evaluated against gaseous cyanide (HCN). This form of cyanide is probably the most likely to be delivered as a chemical warfare agent. It appears that military relevance would require the accumulation of information in developing a militarily suitable and effective antidote for cyanide. Preferably, this antidote would be innocuous, prophylactic in its action and orally administrable. At this point, alpha ketoglutaric acid is the only effective antidote which exhibits all of these properties.

TABLE 1

EFFECTIVENESS OF ANTAGONISTS TO LETHALITY OF CYANIDE IN MICE

ANTAGONIST	DOSE (ANTAGONIST) mg/kg	mM/kg	DOSE (NaCN) mg/kg		mM/kg	mM RATIO	LETHAL %
Kelocyanor*	182.0	0.45	4.5	(LD40)	0.09	5:1	0
Hydroxocobalamin	620.0	0.45	4.5		0.09	5:1	0
Sodium nitrite	30.9	0.45	4.5		0.09	5:1	0
a-Ketoglutaric acid	85.2	0.45	4.5		0.09	5:1	0
Sodium thiosulfate	111.3	0.45	4.5		0.09	5:1	0
Kelocyanor*	182.0	0.45	5.5	(LD70)	0.11	4:1	0
Hydroxocobalamin	620.0	0.45	5.5		0.11	4:1	0
Sodium nitrite	30.9	0.45	5.5		0.11	4:1	0
a-Ketoglutaric acid	85.2	0.45	5.5		0.11	4:1	0
Sodium thiosulfate	111.3	0.45	5.5		0.11	4:1	0
Kelocyanor*	91.0	0.22	5.5		0.11	2:1	0
Sodium nitrite	15.5	0.22	5.5		0.11	2:1	0
a-Ketoglutaric acid	42.6	0.22	5.5		0.11	2:1	0
Sodium thiosulfate	55.7	0.22	5.5		0.11	2:1	0
a-Ketoglutaric acid	551.0	2.90	7.1	(LD90)	0.15	20:1	0
Sodium thiosulfate	719.0	2.90	7.1		0.15	20:1	0
Sodium nitrite	200.0	2.90	0.0		0.00	20:0	100

* Kelocyanor - dicobalt edetate

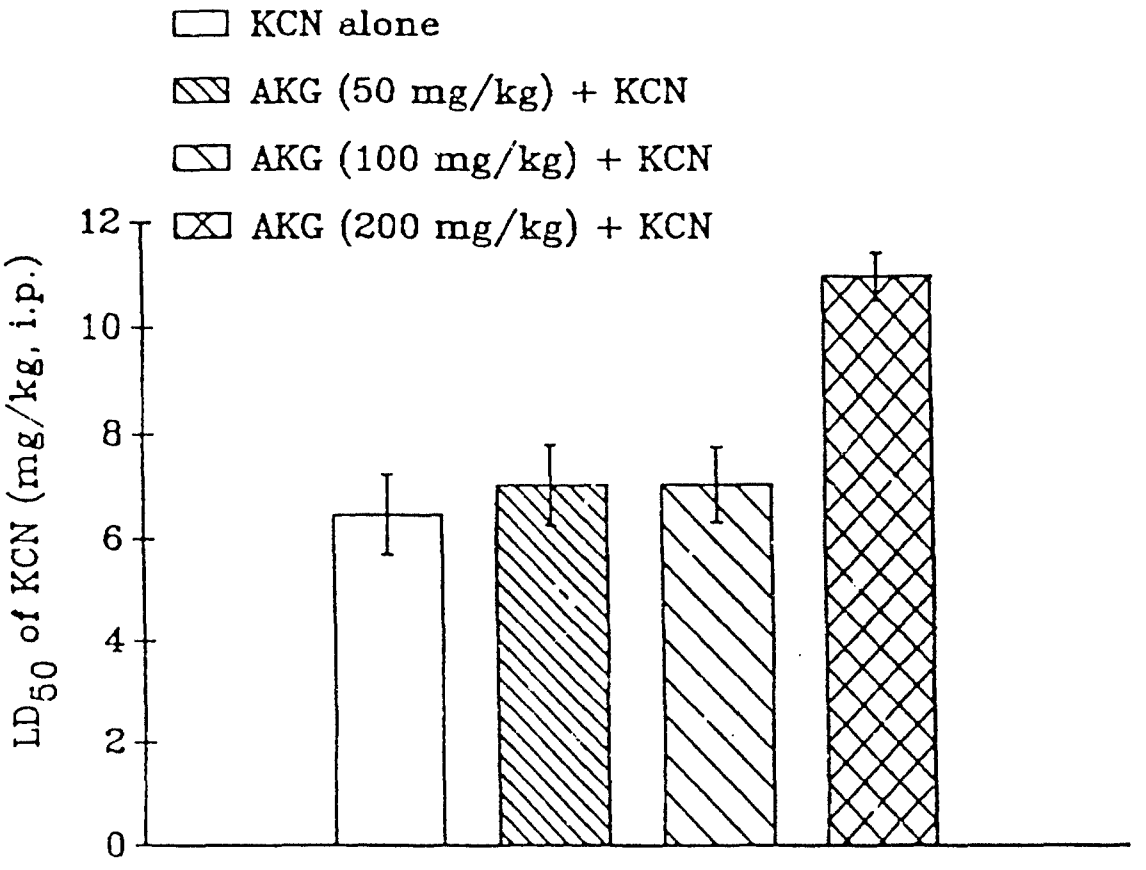


Fig. 3: The efficacy of various doses of AKG to increase the LD_{50} of KCN in mice. Bars represent the 95% confidence limits.

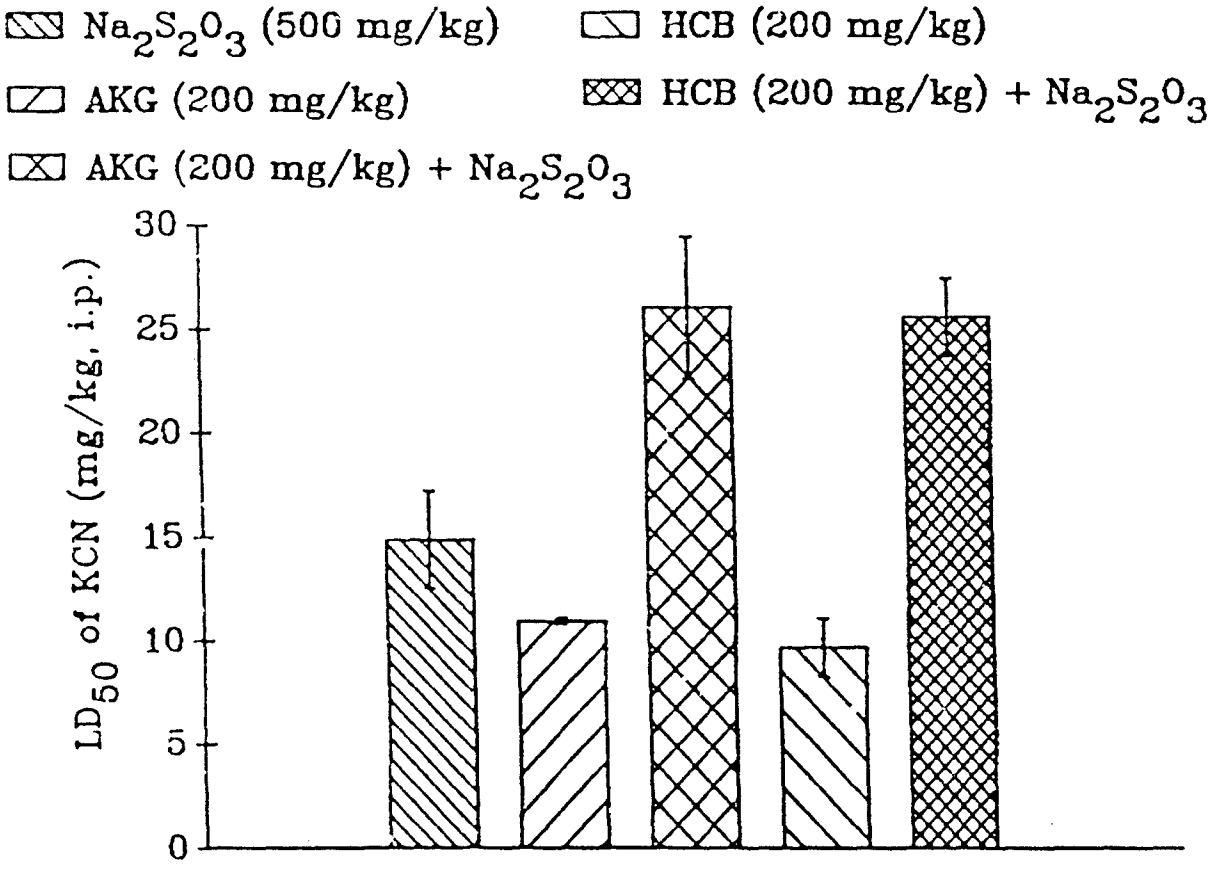


Fig. 4: The efficacy of AKG and HCB alone or in combination with $Na_2S_2O_3$ to increase the LD_{50} of KCN in mice. Bars represent the 95% confidence limits.

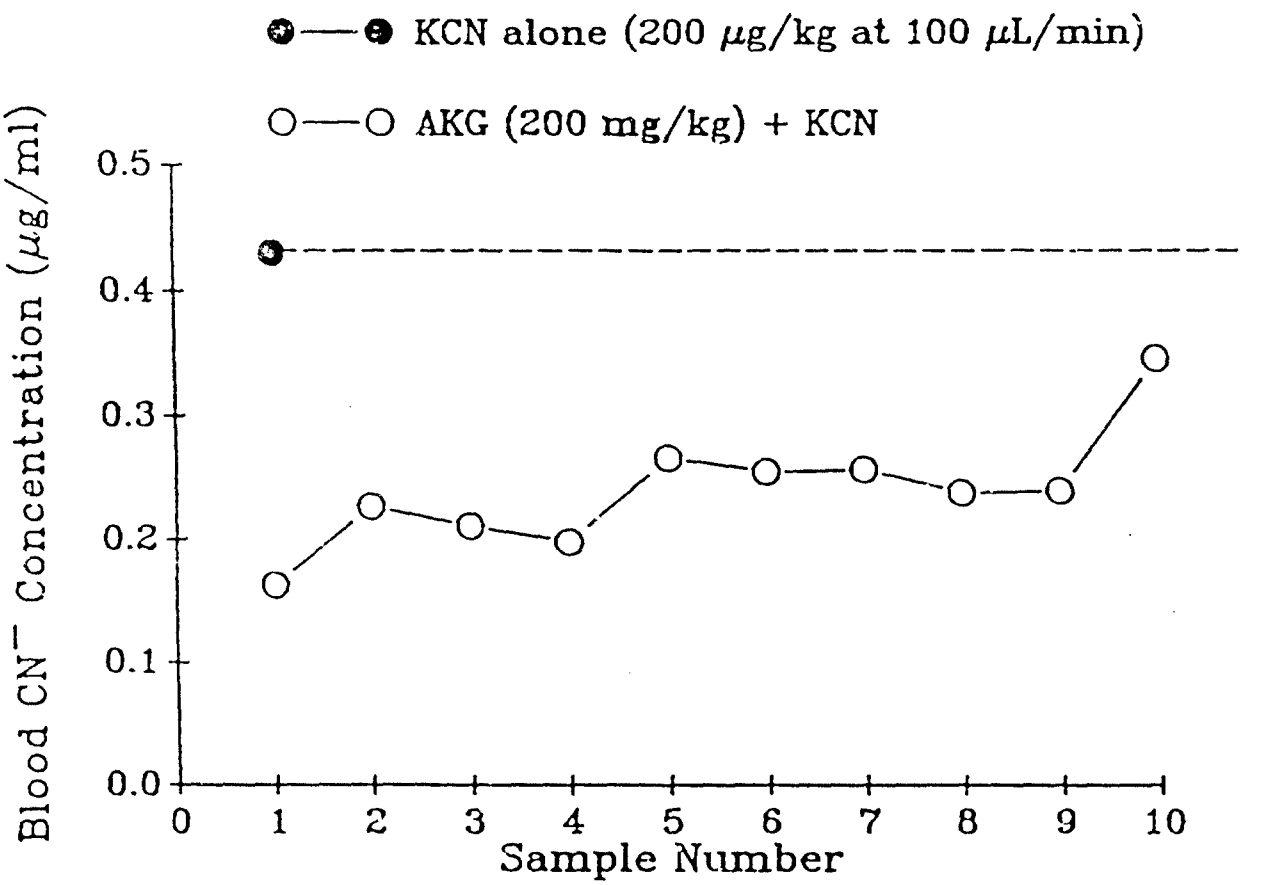
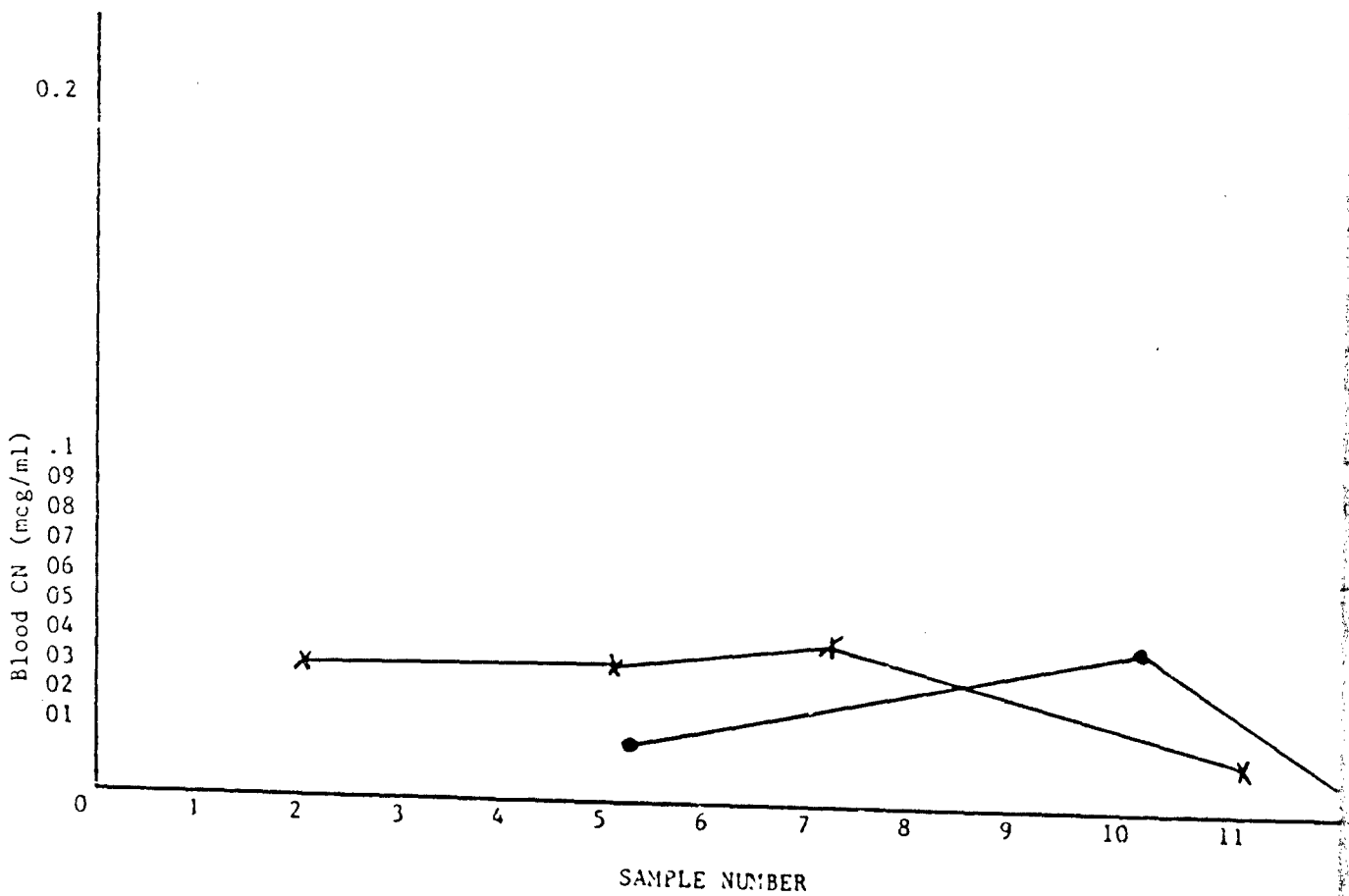


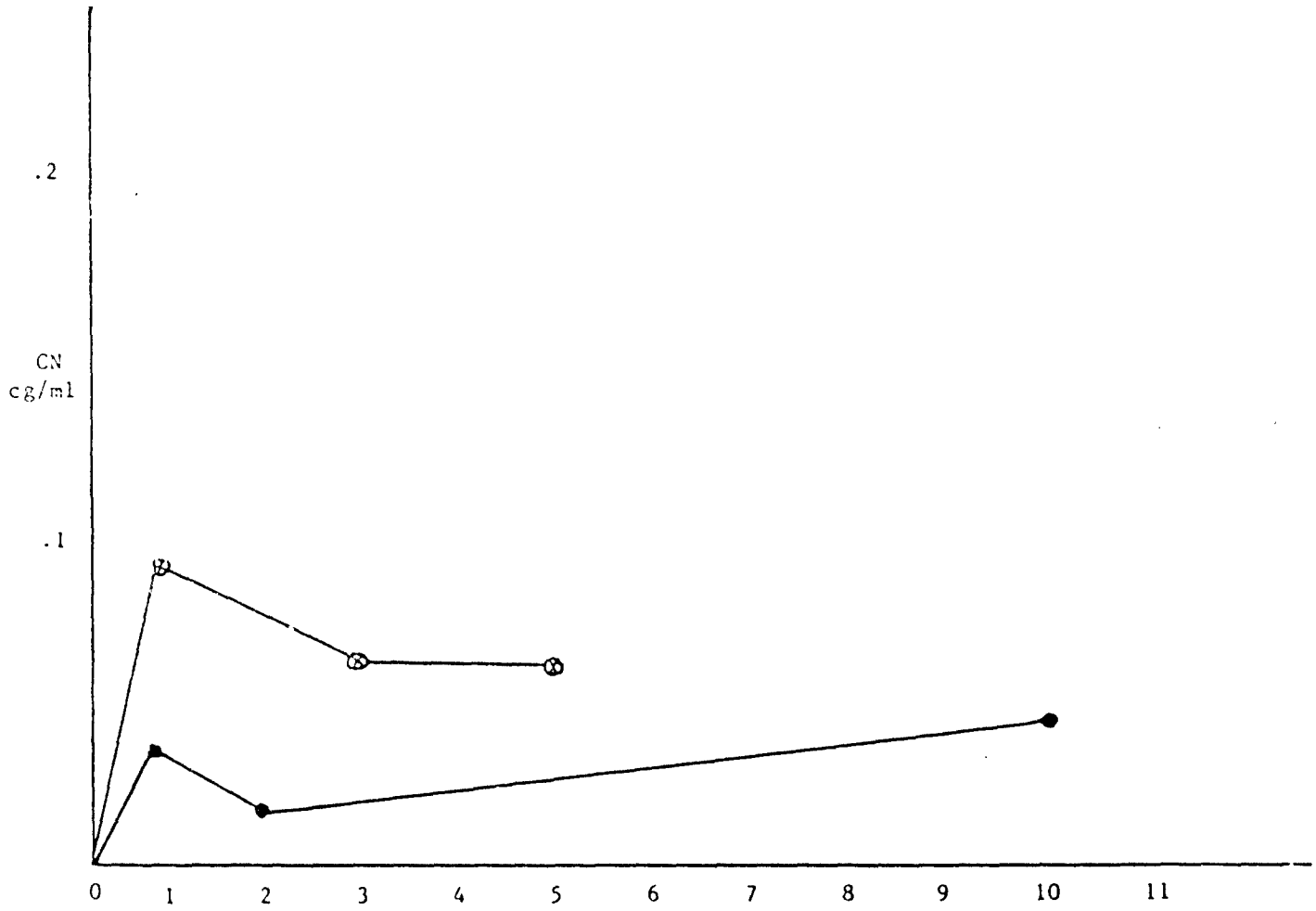
Fig. 5: Blood CN^- levels at approximately 1-min intervals during a constant KCN infusion with and without a bolus dose of AKG.



Rat 1 (•) 0.5mg/kg/0.5ml/22sec

Rat 2 (x) 2.0mg/kg/0.5ml/22sec

Fig. 6: Blood Cyanide Concentrations after Intravenous Cyanide (0.5 mg/kg)



Rat 1 (⊗) 1.0mg/kg i.v./0.5ml/22sec

Rat 2 (●) 1.0mg/kg i.v./0.5ml/22sec

Fig. 7: Blood Cyanide Concentrations after Intravenous Cyanide (1 mg/kg)

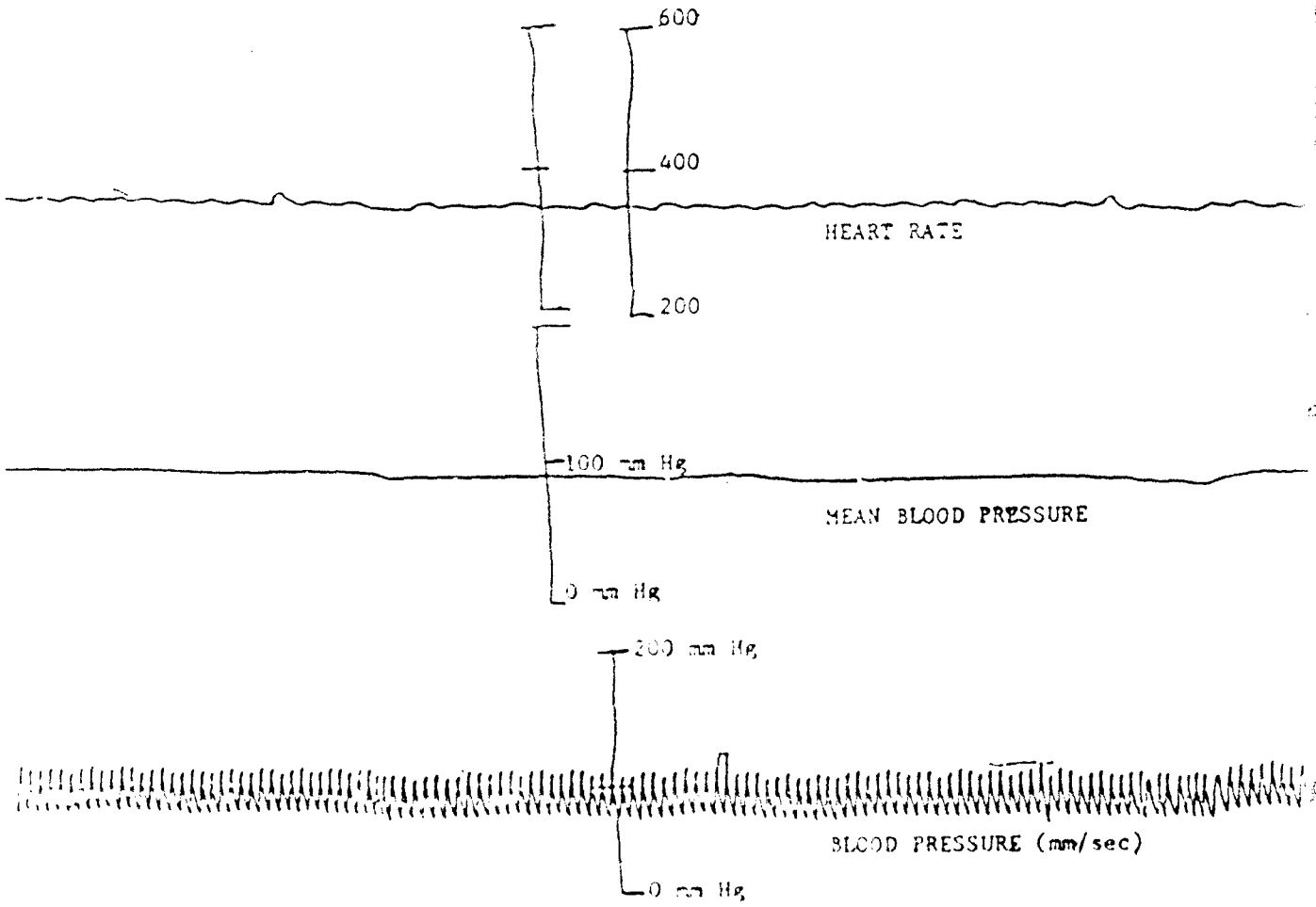


Fig. 8: RECORDING OF BLOOD PRESSURE AND HEART RATE IN CONSCIOUS RATS.

These recordings were obtained from an animal, conscious and catheterized. Such a procedure will be used to determine the effects of cyanide and antidotal activity in animals exposed to HCN in a nose-only chamber.

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